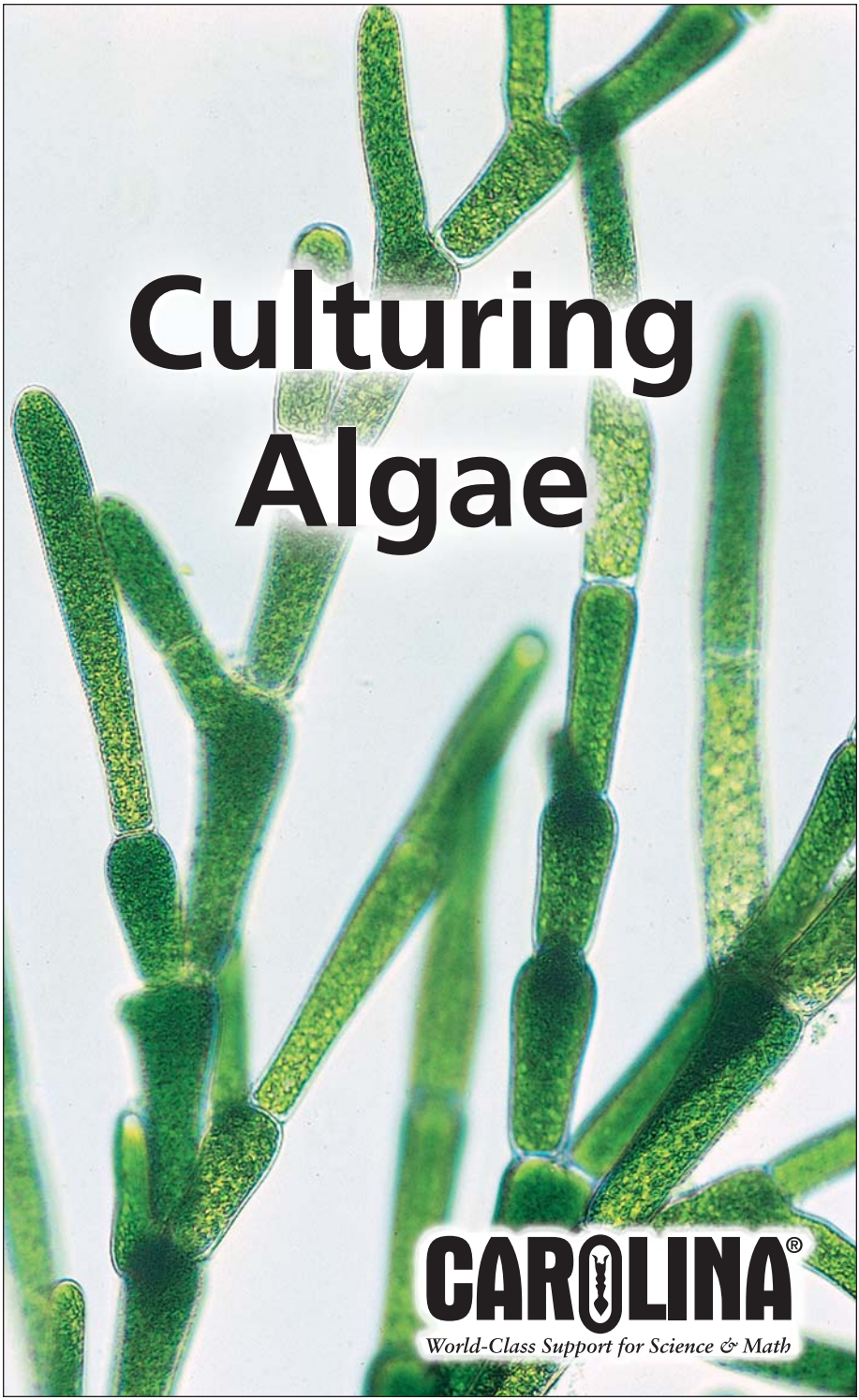
Culturing
Algae
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Culturing Algae

CONTENTS

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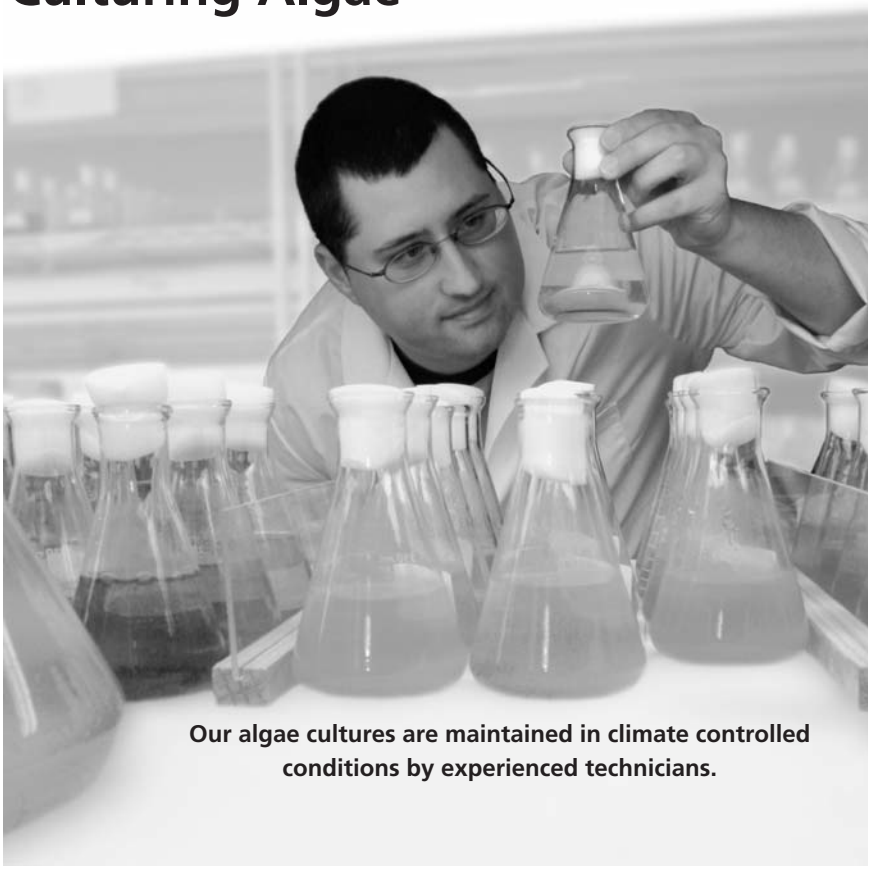
Culturing Algae



Our algae cultures are maintained in climate controlled conditions by experienced technicians.

As with any diverse group of organisms, algae vary in their requirements, demanding different media and different handling techniques from culture to culture. This is compounded when you want a specific culture to do a specific thing at a specific time in the classroom. Even so, using the basic techniques employed with algae, you will find this group of organisms one of the simplest to handle, requiring very little sophisticated equipment, yet providing a wealth of teaching material to demonstrate a wide range of biological principles.

Selecting the correct culture medium for the alga you wish to culture is the key to success. Although a bewildering variety of culture media have been described in the literature, we will concentrate on those that have the widest

applicability for general use. See the Media section for recipes and instructions for preparing these media and recommendations for their use.

A culture tube ready to ship

Our Cultures

Our unialgal cultures are shipped in culture tubes. Most algae are shipped in liquid media, about 10 mL per tube. (A very few, e.g., mating strains of *Chlamydomonas*, are shipped on agar media and are bacteria-free.) Each tube is labeled with the genus of the algae contained and the medium used. Each tube contains enough material to enable up to 30 students to make a slide from the culture and view it under magnification. We do not quantify the algae by means of cell density counts or other methods. Cultures are shipped to be in prime condition when received.

151216 Algae Survey Mixture with identification key

In addition to unialgal cultures, we also supply algae mixtures, which are shipped in culture jars. Mixtures allow students to view a variety of algae quickly and gain experience in identifying different algae through the use of keys and guides. We do not culture mixtures of algae but make each mixture by drawing samples from our unialgal cultures and mixing them before shipment. We do not recommend culturing mixtures because one or two species eventually take over. (For this reason, mixtures are sometimes used in ecological and population studies.)

Although this manual focuses on cultured algae, we also offer collected algae, both freshwater and marine. Collected algae should be used as soon after receipt as possible. Cold-water algae such as *Fucus* (151415) and *Porphyra* (151425) can be held under refrigeration for several days. Tropical forms like *Acetabularia* (151448) should not be refrigerated but must remain at room temperature.

Receiving a Culture and Immediate Care

The most important thing to do after receipt of a culture is to open the shipping container as soon as possible. If left in the packaging away from light, algae will soon die. Loosen the cap and set the culture tube upright in a tube rack or beaker and keep it in a cool area in normal room lighting (not in direct sunlight). In most cases no further care is needed. The culture is best used within 3 days of receipt. If you must hold the culture for more than 5 days, transfer it to a weak nutrient solution such as sterile springwater (or seawater for marine forms) or half-strength Alga-Gro® Medium. This will keep the material in prime condition for classroom use. If you wish to maintain a culture for more than 2 or 3 weeks, or if you wish to expand a culture, refer to the Short-term Culturing section in this manual.

Sterilization

For short-term or long-term culturing, the importance of sterilizing all material that comes in contact with an algal culture cannot be overstressed. Sterilize all glassware, pipets, and media before use by autoclaving for 15 minutes at 15 pounds of pressure. In certain instances (e.g., seawater, which precipitates when autoclaved), filter sterilization should be used instead. If neither steam nor filter sterilization is available, pasteurize the medium by heating it to 73°C and holding it there for 10 to 15 minutes on 2 consecutive days.

Setting Up a New Culture

Once you have the stock cultures you wish to maintain, you have selected and prepared the media, and you have sterilized all the equipment, the next step is the actual transfer and maintenance of the algae cultures. To set up a new culture (a subculture), transfer about 10 mL of a stock culture into 200 mL of fresh medium. Place the new culture under cool-white fluorescent lights (200 to 400 foot-candles) for 7 to 10 days to allow the alga to grow. Once there is good growth, move the culture to an area of lower illumination (50 to 100 foot-candles) for slower growth and storage. If you need a culture for classroom use (short-term culturing), start a new subculture between 10 days and 1 month after setup. If you are establishing a long-term culture, you do not need to subculture again for 3 months to a

year, depending on the particular culture. A general rule to follow for long-term culturing is that flagellates should be transferred every 3 to 4 months and filamentous or unicellular non-motile genera should be transferred every 6 months to a year.

Short-term Culturing

To produce cultures suitable for classroom use, we recommend short-term culturing techniques. Different media are best suited for particular uses of the algae. Take care to assure that the medium you intend to use fits your specific needs. Media such as Soil–Water Medium with its variations and Fishmeal Medium are good for obtaining cultures with normal morphology, but often growth in these media is slower than in other media. For fast-growing, dense cultures to be used in physiology experiments or other activities in which morphology is not a concern, enrichment variations of Bold's Basic Medium, Closterium Medium, or Alga-Gro® Medium will work well. In general, to produce algae of normal morphology, use a dilute medium low in enriching organics—slow growth is often a prerequisite for normal morphology.

Many people prefer to receive their cultures several weeks before the scheduled classroom use and to carry the algae through one or two subcultures. This ensures that the material is in prime condition for class and is available when needed. If you take this approach, try to use the same medium for subculturing that the algae have been growing in. Many algae require several subcultures in a new medium before they adjust physiologically to the nutritional differences. This is especially true in the case of Soil–Water Medium—a major fault of an otherwise excellent medium. Different soils from different sources vary in chemical composition, and a long lag in growth often results from a soil change.

Long-term Culturing

Use long-term culturing techniques to carry stock cultures over extended periods and to maintain a specific culture in the laboratory for future use. For this, you must select a medium that will keep a culture viable for 3 months to a year without the need for continual subculture. Algae from long-term cultures are not usually suitable for classroom use and must be subcultured

in fresh medium or in a different medium for short-term growth. About 3 or 4 weeks before they are needed for classroom use, they should be subcultured to the appropriate medium for short-term growth. About a week before class, they should be subcultured again.

Agar-based media are best for long-term culturing. Bold's basic agar with proteose peptone added does well for many of the green algae, and Allen's modified blue-green agar supports a wide range of blue-green algae (cyanobacteria). One disadvantage of using agar for long-term cultures is that the cultures must be axenic (bacteria-free). For bacterized unialgal cultures and those that will not grow on agar, Soil–Water Medium or one of its variants is good. Another way to make a good long-term medium is to add a layer of liquid (freshwater or seawater) over agar that contains the appropriate nutrients.

By choosing a good medium for long-term culturing, lowering the temperature 5 to 8°C below that needed for short-term culturing, and decreasing illumination by about half, you greatly reduce the work of maintaining a permanent collection of algae for teaching.

Media

It is good to become familiar with a number of the more commonly used media and their variations in regard to the types of cultures they produce. Formulas and instructions for preparing these media are given below.

Alga-Gro® Freshwater Medium is available in bottles or in tubes of concentrate.

Freshwater Media

Alga-Gro® Freshwater Medium is a defined, dilute medium designed in our laboratory for short-term growth of a large variety of algae for normal morphology. The standard medium is used at a pH of 7.8 for most algae, but for some blue-green algae we adjust the pH to 6.5. This medium was

originally designed for use with springwater, but an adequate medium can be made using distilled water.

Alga-Gro® is available both as a ready-to-use, full-strength medium (153752) and as a concentrate (153751) that must be diluted before use. For each liter of medium required, add 20 mL (one tube) of Alga-Gro® Concentrate to 1 L of springwater or glass-distilled water. Adjust the pH to 7.8 using 0.5 N KOH and 0.5 N HCl to back-titrate if necessary. Dispense into containers and autoclave at 15 pounds pressure for 15 minutes.

Allen's Blue-Green Medium (Modified) is a good basic medium for a wide variety of blue-green algae. We have found that several blue-green algae such as *Gloeocapsa* (151800) do best on this medium.

For a liter of medium, add the following to 999 mL of glass-distilled water:

$NaNO_3$	1.59 g
K_2HPO_4	0.039 g
$MgSO_4 \bullet 7H_2O$	0.075 g
Na_2CO_3	0.02 g
$Ca(NO_3)_2 \bullet 4H_2O$	0.02 g
$Na_2SiO_3 \bullet 9H_2O$	0.058 g
EDTA	0.001 g
Citric Acid	0.006 g
$FeCl_3$	0.002 g
Micronutrients*	1 mL

**Micronutrients.* To 1 L of glass-distilled water, add the following:

H_3BO_4	2.86 g
$MnCl_2 \bullet 4H_2O$	1.81 g
$ZnSO_4 \bullet 7H_2O$	0.222 g
$Na_2MoO_4 \bullet 2H_2O$	0.391 g
$CuSO_4 \bullet 5H_2O$	0.079 g
$Co(NO_3)_2 \bullet 6H_2O$	0.0494 g

Adjust the pH to 7.8 and autoclave at 15 pounds of pressure for 15 minutes. For solid medium, equal volumes of double-strength salt solution and double-strength agar are separately sterilized and combined at 48ºC.

Bold's Basic Medium is an improvement of the old Bristol's Medium and is good for long-term culturing.

Prepare six different stock salt solutions, each containing one of the following salts in the amount listed:

$NaNO_3$	10 g/400 mL H_2O
$MgSO_4 \bullet 7H_2O$	2 g/400 mL H_2O
K_2HPO_4	4 g/400 mL H_2O
KH_2PO_4	6 g/400 mL H_2O
$CaCl_2$	1 g/400 mL H_2O
NaCl	1 g/400 mL H_2O

To 936 mL distilled water, add 10 mL of each stock salt solution and 1.0 mL each of the following micronutrient solutions:

A. *EDTA Stock.* To 1 L glass-distilled water, add 50 g EDTA and 31 g KOH (85%).

B. *H-Fe Stock.* To 1 L acidified water*, add 4.98 g $FeSO_4 \bullet 7H_2O$.

C. *Boron Stock.* To 1 L glass-distilled water, add 11.42 g H_3BO_3.

D. *H-H5 Stock.* To 1 L acidified water*, add the following:

$ZnSO_4$	8.82 g
MoO_3	0.71 g
$Co(NO_3)_2 \bullet 6H_2O$	0.49 g
$MnCl_2$	1.44 g
$CuSO_4 \bullet 5H_2O$	1.57 g

**Acidified Water.* To 999 mL of glass-distilled water, add 1 mL concentrated H_2SO_4.

Dispense into vessels and sterilize by autoclaving for 15 minutes at 15 pounds pressure. If solid medium is desired, add 15 g agar per liter of medium.

Several variations of Bold's Basic Medium help encourage the growth of certain fastidious algae. Here are three of the most useful.

a. To each liter of medium, add 1 g proteose peptone.

b. To 500 mL half-strength medium, add 500 mL soil–water supernatant.

c. To each liter of medium, add the following enrichments:

Yeast extract	0.5 g
Sodium acetate	0.1 g
Vitamin B_{12}	1.0 µg

Bristol's Medium, one of the simplest of the basic salts media, can be made with many variations. The basic medium supports a number of the more hardy algae and is good for long-term culturing. The formula for this medium is the same as Bold's Basic Medium, but 50 mL of soil-water supernatant (153790) is usually added to each liter of medium in place of adding the micronutrient solutions.

Closterium Medium yields promising results for a number of algae, especially desmids. It gives high volume yields, but we have noticed some abnormal morphology in some cultures.

For each liter of medium, add the following to 997 mL glass-distilled water:

$Ca(NO_3)_2 \bullet 4H_2O$	0.15 g
KNO_3	0.10 g
$MgSO_4 \bullet 7H_2O$	0.04 g
$C_3H_7Na_2O_6P \bullet xH_2O$	0.05 g
Vitamin B_{12}	0.1 µg
Biotin	0.1 µg
Thiamine HCl	10.0 µg
P IV Metals*	3.0 mL
Tris Buffer	0.5 g

Adjust pH to 7.5

**P IV Metals*. To 500 mL glass-distilled water, add 0.750 g Na2 EDTA. After this chelating agent has dissolved, the following salts are added in the amounts indicated:

$FeCl_3 \bullet 6H_2O$	97 mg
$MnCl_2 \bullet 4H_2O$	41 mg
$ZnCl_2$	5 mg
$CoCl_2 \bullet 6H_2O$	2 mg
Na_2MoO_4	4 mg

EREM Medium (153765) is the "b" variation listed under Bold's Basic Medium. This medium gives good morphology and growth with such algae as *Pandorina*, *Eudorina*, and *Batrachospermum*.

Fishmeal Medium is a very dilute medium that gives good morphology in cultures of *Hydrodictyon* and our large-form *Chlamydomonas*. It also supports growth of a number of the colonial Volvocales.

Stock Solutions:

A. Fishmeal–Soil stock: To 2 L water, add ¼ teaspoon fishmeal and 2 teaspoons soil. Bring to a boil, cool, and filter. Store in the refrigerator.

B. $FeCl_3$ Stock: To 100 mL water, add 0.1 g $FeCl_3$.

Add 100 mL of the fishmeal–soil stock solution and 5 mL of the $FeCl_3$ stock solution to 1000 mL of springwater. Dispense into vessels and autoclave at 15 pounds pressure for 15 minutes.

Chlamydomonas Medium (153760) is a slight modification of Fishmeal Medium.

Soil–Water Medium with its variations is excellent for long-term culturing and normal morphology. So far, we have found no substitute for Soil–Water Medium for culturing *Merismopedia* (151835) and *Spirogyra* (152525) when normal morphology is required. The medium supports a very wide range of algae, but an inherent problem is finding a reliable source of soil. One answer to the problem of a soil source was suggested by Dr. Richard Starr. He found used soil from the Indiana University greenhouses to be a very stable soil source. Those greenhouses dumped all their used soil in one outdoor area and allowed it to stand for about a year before reusing it in the greenhouses. Dr. Starr found that after this soil had aged for between 6 months and a year it made a dependable soil–water medium. We offer a Soil–Water Medium (153785) and a concentrated Soil–Water extract (153790). If you have a source of suitable garden soil as noted above, you can make your own as follows:

Place a pinch of $CaCO_3$ in the bottom of the culture vessel and cover it with about ½ inch of good garden soil. The soil should be of about medium humus content and should not contain commercial fertilizer. You may successfully use any good potting soil, but be sure to avoid mixes that contain high concentrations of peat or sphagnum.

After adding the ½ inch of soil, fill the vessel three-fourths full with glass-distilled, spring-, or pond water and either plug it or cap it. The vessel is then steamed (not autoclaved) for 2 hours on 2 consecutive days. The steaming may be done in a pressure cooker or autoclave by allowing the steam to escape continually through the exhaust. This medium is very good for nonsterile cultures where normal morphology is desired.

Spirogyra (152525) and *Zygnema* (152695) do best in Soil–Water Medium if the pinch of $CaCO_3$ is omitted.

For acid-thriving cultures, omit the $CaCO_3$ and add a small amount of acid peat to the soil.

Some algae, such as *Euglena* (152800), seem to require complex nitrogenous or carbon compounds. For these organisms, adding one-fourth or one-half of a common garden pea to the medium greatly enhances growth.

Many brackish and saltwater forms, especially the marine flagellates, grow very well in Soil–Water Medium if natural seawater or dilutions of it (e.g., quarter to half strength) are substituted for the distilled water and if the $CaCO_3$ is omitted.

Volvox Medium. For each liter of medium required, add stock solutions in the amounts indicated to 981 mL of glass-distilled water:

1 mL $Ca(NO_3)_2 \bullet 4H_2O$	11.8 g/l00 mL
1 mL $MgSO_4 \bullet 7H_2O$	4.0 g/ 100 mL
1 mL $C_3H_7Na_2O_6P \bullet xH_2O$	5.0 g/100 mL
1 mL KCI	5.0 g/l00 mL
10 mL Glycylglycine	5.0 g/l00 mL
1 mL Biotin	25.0 µg/l00 mL
1 mL Vitamin B_{12}	15.0 µg /l00 mL
3 mL P IV Metals	(see Closterium Medium)

Adjust pH to 7.0 with 1 N NaOH.

Saltwater Media for Marine Algae

Alga-Gro® Seawater Medium is the same formula as used with freshwater algae, but with aged natural seawater substituted for springwater. This medium is excellent for the larger marine algae but gives good growth with only a limited number of marine flagellates.

For each liter of medium required, pasteurize* 1 L natural seawater by slowly heating to 73°C on 2 consecutive days. When the seawater has cooled after the second pasteurization, add 20 mL (one tube) of Alga-Gro® Concentrate (153751). Mix the solution and dispense into sterile containers.

*In place of pasteurizing, you may sterilize the seawater by autoclaving at 7 pounds pressure and 110°C for 3 minutes.

Erdschreiber Medium was one of the early successful marine media and is still good for a great many algae. Its major disadvantage is that it uses soil–water supernatant, which, as pointed out earlier, requires a good soil source (or you may use our 153790 Soil–Water Supernatant). This medium not only produces good cultures of the larger marine algae but also supports growth of many of the marine flagellates.

To 1 L pasteurized or sterilized seawater, add 50 mL sterilized soil–water supernatant and 20 mL sterilized salts solution.

Salts Solution: To 200 mL glass-distilled water, add the following:

$NaNO_3$	2 g
Na_2HPO_4	0.3 g
Vitamin B_{12}	15 µg

Guillard's Medium. We prefer either our own Alga-Gro® Seawater Medium or Provasoli's E. S. Medium for culturing, but have added this one to the list because it is used extensively and is a very good medium.

To 1 L sterilized or pasteurized seawater, add 20 mL sterile salt solution and 10 mL trace metal solution:

Salt Solution: To 200 mL distilled water, add the following:

$NaNO_3$	1.5 g
$NaH_2PO_4 \bullet H_2O$	0.1 g
Fe Sequestrene	0.1 g

$Na_2SiO_3 \bullet 9H_2O$	0.1 g
Thiamine HCl	0.002 g
Biotin	1 µg
Vitamin B_{12}	1 µg
Tris Buffer	5 g

Trace Metal Solution: To 1 L distilled water, add the following salts:

$CuSO_4 \bullet 5H_2O$	0.019 g
$ZnSO_4 \bullet 7H_2O$	0.044 g
$CaCl_2 \bullet 6H_2O$	0.20 g
MnCl	0.36 g
$Na_2MoO_4 \bullet 2H_2O$	0.0126 g

Provasoli's ASP 6 Medium (Modified) is a completely synthetic medium that supports a limited number of marine algae. When culturing classroom material, we recommend using one of the media that uses natural seawater. Most of the synthetic media we have tried produce cultures with some degree of abnormal morphology.

For each liter of medium required, stock solutions in the amounts indicated are added to 814.50 mL of glass-distilled water.

100 mL NaCl	240 g/l000 mL H_2O
20 mL $MgSO_4 \bullet 7H_2O$	80 g/200 mL H_2O
10 mL KCl	7 g/l00 mL H_2O
10 mL $CaCl_2$	1.5 g/l00 mL H_2O
10 mL $NaNO_3$	3 g/l00 mL H_2O
10 mL $C_3H_7Na_2O_6P \bullet xH_2O$	1 g/l00 mL H_2O
10 mL Tris Buffer	10 g/100 mL H_2O
3.5 mL Vitamin B_{12}	25 µg/166 mL H_2O
5 mL Biotin	10 µg/100 mL H_2O
1 mL $Na_2MoO_4 \bullet 2H_2O$	0.05 g/100 mL H_2O
1 mL Thiamine	0.0005 g/l00 mL H_2O
5 mL P II Metals*	

**P II Metals.* Add the following to 100 mL distilled water:

H_3BO_3	114 mg
$FeCl_3 \bullet 6H_2O$	4.9 mg
$MnSO_4 \bullet 4H_2O$	16.4 mg

$ZnSO_4 \bullet 7H_2O$	2.2 mg
$CoSO_4 \bullet 7H_2O$	0.48 mg
Na_2EDTA	100 mg

Provasoli's E. S. Medium is an excellent medium, giving good growth for a wide range of marine algae.

To 100 mL glass-distilled water, add the following:

$NaNO_3$	350 mg
$C_3H_7Na_2O_6P \bullet xH_2O$	50 mg
Fe (as EDTA 1:1 M)*	2.5 mg
P II Metals**	25 mL
Vitamin B_{12}	10 µg
Thiamine	0.5 mg
Biotin	5 µg
Tris Buffer	500 mg

Adjust pH to 7.8.

Add 20 mL of the above enrichment to 1000 mL filtered seawater. For bacteria-free cultures, autoclave the enrichment in tubes and add aseptically to filter-sterilized or autoclaved seawater.

*Fe (as EDTA 1:1 M). Dissolve 351 mg $Fe(NH_4)_2(SO_4)_2 \bullet 6H_2O$ and 300 mg Na_2EDTA in 500 mL H_2O. One mL of this solution provides 0.1 mg Fe.

**P II Metals.* See Provasoli's ASP 6 Synthetic Medium.

Soil–Seawater Medium is a variation of the Soil–Water Medium mentioned earlier. Substitute seawater for the freshwater used in the soil-water formulation. This medium is not particularly good for larger seaweeds but is excellent for marine flagellates. It may also be used to culture brackish water algal forms such as *Spirulina* (151900), by mixing it with distilled water in dilutions of quarter, half, or three-quarters strength, depending on what is needed.

Water

One of the important aspects of any culture medium is the water source. We use either aged natural waters or glass-distilled water when preparing culture media. Normal tap water, deionized water, and distilled water from metal stills often contain enough toxic material to cause problems in culturing. This is also true of natural waters that have not been aged or

treated with charcoal. The preferred method is to age natural seawater for at least 6 months in the dark at 5°C, and to age springwater and lake water for about 2 months in the dark at room temperature (about 20°C).

A second method of treating natural waters is with activated charcoal. Add 2 g activated charcoal (USP Grade) per liter of water, agitate 1 hour, and filter. This allows use of freshly collected water without the bother of aging. Some deionization companies run their water through activated charcoal as part of the purification process, and this product has been used successfully for algae culture.

Because of the expense of natural seawater to inland schools, some laboratories have tried using the commercial seawater preparations designed for marine aquaria. We have tried several brands and found most of them rather disappointing. Their efficiency seemed to depend to an extent on how they were sterilized. Poorest growth results were obtained when the media were autoclaved. Pasteurization gave somewhat better growth results, with filter sterilization showing the best growth. Even with filter sterilization of the media, many cultures did not grow as well as with natural seawater, and they showed varying degrees of abnormal morphology. Where possible, use natural seawater for culturing.

Illumination

We recommend using cool-white fluorescent lights for culturing algae. Incandescent bulbs or direct sunlight can cause problems because of the heat that they generate. Cultures grown in direct sunlight often reach a temperature as much as 10°C higher than that of the room. If you use sunlight for culturing, diffuse the rays by covering the window with tissue paper or the shading paint used for greenhouses.

For short-term culturing, most of our algal cultures require a temperature of 22°C and a high light level of 200 to 400 foot-candles provided by fluorescent tubes or bulbs placed 45 to 60 cm (18 to 24") above the culture. Our catalog and online listings give recommended light levels and temperatures for each culture.

Most cultures do best when given a dark period each day. Adjustable, inexpensive time clocks are available that turn culture lights on and off on

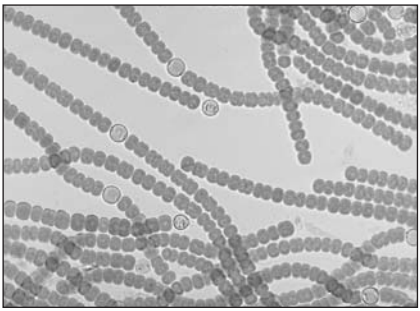
a fixed schedule. The two most used time sequences are 12 hours light followed by 12 hours dark and 16 hours light followed by 8 hours dark. Our standard practice is to use a 16 light:8 dark cycle for our short-term cultures and a 12 light:12 dark cycle for our stock long-term cultures.

Temperature

Most freshwater and Atlantic coast marine algae grow well between 15° and 25°C. Generally, the lethal temperature for these algae is somewhere above 30°C. Marine algae from the Pacific coast prefer cooler temperatures—between 5° and 15°C. Some of them cannot survive temperatures above 20°C. While controlled temperatures are preferable for growing algae, most of our cultured algae do well at room temperature and will even tolerate reasonable swings in temperature of 10° or so during a 24-hour period.

Requirements of Specific Algae

Cyanobacteria (Blue-green Algae)



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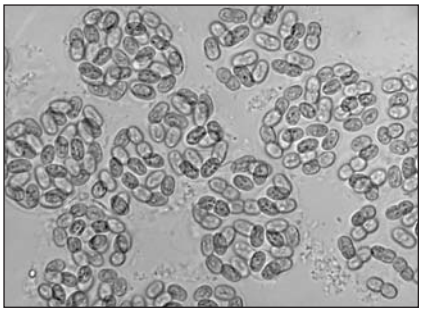


Gloeocapsa

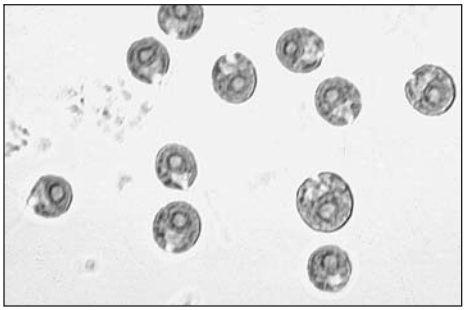
Cyanobacteria tend to bleach easily under high light intensities. For this reason, we place our initial subcultures in an area where they get only about 100 foot-candles of light. Certain blue-green algae such as *Spirulina* (151910) never do well under direct light and should be kept at very low light intensities at all times. Generally, when culturing for morphology, the blue-green algae do best in Soil–Water Medium. This is especially true of *Merismopedia* (151835) and *Gloeocapsa* (151800). Recommended temperature is 22°C.

Green Algae

The green algae are a diverse group in terms of culture requirements. Most of these algae can be grown in either Soil–Water Medium or Alga-Gro® Medium. Most require a high light level (200 to 400 foot-candles) and a temperature of 22ºC for best growth.

Chlamydomonas

The large form of *Chlamydomonas* (152030) tends to become palmelloid when grown in rich media. It should be cultured in either Fishmeal Medium or Soil–Water Medium to keep the cells motile.



Chlamydomonas

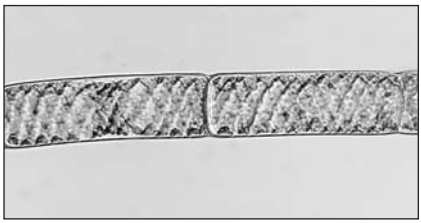
We also have mating strains (+ and –) of *Chlamydomonas moewusii* (152034 and 152035) and *Chlamydomonas reinhardi* (152040 and 152041). These are shipped on agar slants and are bacteria-free. The 151610 Chlamydomonas Mating Kit comes with two mating strains, mating solution, and instructions.

Chlorella

The unicellular, nonmotile, freshwater alga *Chlorella* (152069) was used as a model organism by Calvin in his studies of photosynthesis. Current research focuses on its potential as a food and energy source. *Chlorella* grows quickly in Alga-Gro® and is easy to culture.

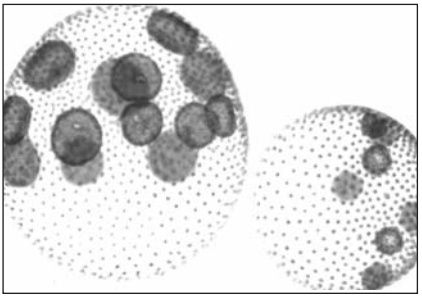
Spirogyra and Zygnema

Spirogyra (152525) is difficult to grow in any media other than Soil–Water Medium (minus the calcium carbonate) and still get normal morphology. *Spirogyra* produces cells with abnormal plastids in most other media. *Zygnema* (152695) is cultured in EREM Medium. These algae are known for their distinctive chloroplasts—spiral in *Spirogyra* and stellate in *Zygnema*. Both grow rapidly in culture.



Spirogyra

Volvox and Other Colonial Volcocales



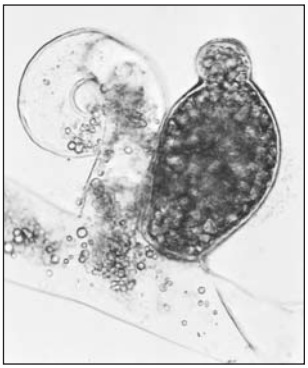
***Volvox,* spherical colony**

For normal morphology, these algae must be transferred about every 10 days. They grow well in Soil–Water Medium, Alga-Gro® Medium, and dilute Bold's Basic Medium with soil-water supernatant added. Some sexual material appears when *Volvox* is grown in Alga-Gro® Medium, but for controlled sexual cultures, they must be grown axenically in Volvox Medium.

Euglenoids

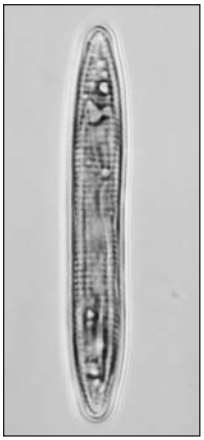
For classroom use, one of the best ways to culture the euglenoids is in Soil–Water Medium with a garden pea added. Dense, axenic cultures of *Euglena gracilis* may be grown in sterile *Euglena* broth. Our preparation for in-house use contains 1 L deionized water, 1 g, sodium acetate, 1 g beef extract, 2 g tryptone, 2 g yeast extract, and 0.01 g calcium chloride.

Yellow-green Algae



Vaucheria

Vaucheria (152995) is grown in Alga-Gro®. It exhibits mitosis without cytokinesis; thus, the filaments are coenocytes and grow from their tips. In nature, *Vaucheria* often forms a dense mat either in water or on stream banks or rock faces that seep water. Culture requirements are similar to those for green algae.



Synedra

Diatoms

Diatoms, such as *Synedra*, grow on a number of different media, but a source of silicon must be provided for continued good growth. Normally, sodium

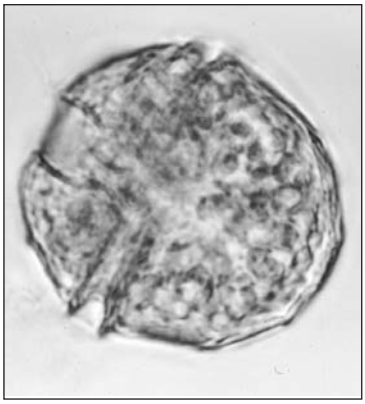
metasilicate ($Na_2SiO_3 \bullet 9H_2O$) will meet this requirement when used in concentrations of 10 to 30 mg per liter.

Golden-brown Algae

Our cultures demonstrate a variety of characteristics. *Isochrysis* (153180) and *Nannochloropsis* (153220) are unicellular, while *Synura* (153210) forms spherical colonies. *Isochrysis* and *Synura* are flagellated, whereas *Nannochloropsis* is nonmotile. *Isochrysis* is cultured in Soil–Seawater. *Nannochloropsis*is is cultured in Alga-Gro® Seawater Medium. *Synura*, a freshwater genus, is cultured in Soil–Water Medium. Species of *Nannochloropsis* are currently being researched for possible use in biodiesel production. These algae require a high light level (200 to 400 foot-candles) and a temperature of 22°C for best growth.

Dinoflagellates

The marine dinoflagellates *Amphidinium* (153240), *Gymnodinium* (153260), and *Prorocentrum* (153300) are cultured in Alga-Gro® Seawater. They also do well on Erdschreiber Medium. *Peridinium* (153290), a freshwater genus, is cultured in Soil–Water Medium but will also grow well in Alga-Gro® Medium.



Peridinium

The addition of very low concentrations of yeast extract, thiamine, and proteose peptone stimulates dinoflagellate growth in axenic cultures. Dinoflagellates require a high light level (200 to 400 foot-candles) and a temperature of 22°C for best growth.

Brown Algae

The brown algae are hardy growers and will normally grow well in Alga-Gro® Seawater, Erdschreiber, or Provasoli's E. S. media. They require a high light level (200 to 400 foot-candles) and a temperature of 22°C for best growth. Some of the larger brown algae such as *Fucus* and *Laminaria* cannot be

cultured for classroom use; however, collected specimens remain usable for long periods when kept refrigerated.

Red Algae

The red algae are like the cyanobacteria (blue-green algae) in that they cannot tolerate high light intensities. We do not grow ours under regular lights, but keep them at less than 150 foot-candles of light, even during subculturing. Most are grown in Alga-Gro® Seawater, but *Batrachospermum* (15355), which is freshwater, is cultured in EREM Medium.

Ectocarpus

Techniques for Establishing Cultures from Nature

Each species reacts differently. Techniques for isolating and purifying cultures must be adjusted and adapted as needed.

Collection

Acquire your initial supply of algal material from a natural population. Floating and swimming algae can be collected and concentrated efficiently with plankton nets. Attached and filamentous forms can be scraped from rocks, leaves, and larger algae. When you collect specimens directly, observe and begin isolating them within a very short time, as many of the more delicate (and often more interesting) genera may begin to disappear within a few hours.

Another method of collecting specimens is to get small amounts of mud and sand at the water line and to air dry it until the soil feels dry and crumbles easily. When small samples of this dried soil are placed in petri dishes, covered with sterile water or medium, and placed under a light, many different algae and protozoa begin to emerge within 24 hours. Organisms continue to appear in the dishes for several days. Adding a

sterilized barley grain or a pea cotyledon to the dishes often encourages growth of genera that might otherwise go unnoticed.

Algal zygotes, spores, and other resting forms will remain viable for a number of years if dried soil samples are stored in sealed jars or plastic bags.

Isolation

To assure homogeneity, start unialgal cultures from clones. A clone is propagated from a single cell, a single filament of a few cells, or a piece of a thallus. A serious effort to establish a unialgal culture must begin with a clone.

Micropipet Washing Technique

One of the simplest techniques for isolating microscopic forms uses micropipets to carry individual cells through a series of sterile washes.

Either soft glass tubing (3 to 4 mm bore) or disposable glass Pasteur pipets can be used to form a micropipet. To make a micropipet, clasp the tip of a sterilized pipet with a pair of forceps and hold the glass in a small Bunsen burner flame until it begins to soften. In a motion that coincides with the removal of the glass from the flame, stretch the micropipet tip with a smooth, rapid pull. By varying the timing and strength of the pull, you can adjust the bore of the micropipet to the size needed for any microorganism. With forceps, break the tip from the micropipet with a steady, gentle, outward pull in a straight line with the pipet to make a smooth even tip. Bending or crushing the tip produces a jagged edge that is difficult to use. Attach a rubber bulb or piece of soft rubber tubing to the top of the pipet.

While looking through a dissecting microscope, place a small sample of the collection containing the organisms in a depression of a sterile spot plate, a depression slide, or a watch glass. Fill 6 to 12 other depressions with sterile water or media. While watching through the dissecting microscope, move the tip of a micropipet over one of the cells. Carefully dip the micropipet tip into the medium, pick up the cell, and expel it into another depression of sterile water or medium. Isolate at least 12 to 15 single cells in this way and carefully wash them 6 to 12 times in depressions of sterile fluid. Use a new micropipet for each washing.

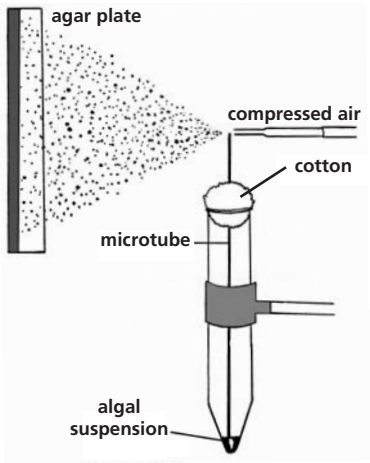
After sufficient washings, expel a single cell into each of several tubes of sterile medium. Place the tubes under lights to stimulate algal growth.

Cultures started from single cell isolates often take 3 to 6 weeks to attain notable growth.

Use a variation of this technique to isolate filamentous algae. Through a procedure similar to making a micropipet, make a small microhook. Carefully, use the hook to lift a single filament of an alga. Follow this by isolation and washing. After careful washing, the algal filament should be pulled through a petri dish of very soft agar (0.5% to 0.75%) several times to help remove any epiphytes.

Atomizer Technique

Wiedeman et al. (1964) developed a technique that can be used to both isolate and purify collections of algae. Wash 8 to 9 mL of algae by centrifugation (see Purification). After the final wash, decant all but 2 mL of the supernatant. Make a 15-cm-long microtube by following the instructions for making a micropipet. Insert the microtube in the bottom of the centrifuge tube and hold it in place with a cotton stopper. Fasten the centrifuge tube to a ring stand and direct a stream of compressed air through a small opening (e.g., through a dropper pipet) across the opening of the microtube, which extends above the mouth of the centrifuge tube. The algal suspension is drawn up the microtube and atomized into a fine spray. Pass a sterile plate of agar medium quickly through the spray at a distance of about 25 cm. Cover the plate and put it under light. After several days' growth, single cells or colonies free of bacteria and fungi can be picked from the surface of the agar with a micropipet and expelled into sterile liquid medium.



Atomizer apparatus

Selective Media Technique

Different methods are required for isolating larger and more complex marine algae. To simplify isolation of vegetative material, use a series of selective media to free an alga from some of the worst contaminants in the collected sample.

Select a branch or piece of the algal body (thallus) that is as clean and as free as possible of epiphytes. While viewing through a dissecting microscope, brush the piece of thallus with a camel's-hair brush or with cotton to free it of as many epiphytes as possible. A microhook is often helpful in removing epiphytes. Wash the thallus several times in sterile seawater and place it in a medium containing 10 mg/L germanium dioxide. Germanium dioxide is a silica antagonist and prevents the growth of diatoms and other siliceous organisms that are the worst contaminators of marine cultures.

Additional Techniques

These additional techniques and variations are sometimes useful.

Agar Pour Plates: Mix a small inoculum of a raw collection with cool, but not yet solidified agar containing a weak nutrient solution. Swirl the mixture before pouring it into a petri dish, where it will harden. After several days of growth under lights, cut colonies from the agar and inoculate into fresh medium.

Taxis: Many motile algae react either positively or negatively to light or to electric current. Also, different genera respond at different rates, which allows for differential separation. Repeated isolations using light or current may produce nearly pure separations of genera.

Osmotic Balance: Some of the hardier algae may tolerate osmotic changes better than their contaminators do. By moving the specimen from distilled water to a weak salt solution and back to distilled water, you may remove many protozoans and naked flagellates.

Reproductive Structures: Isolate resting spores, cysts, zygotes, tetraspores, or other reproductive structures and germinate them to give rise to pure cultures.

Purification

After the unialgal culture has been established and is growing well, many types of research require that the culture be axenic (bacteria-free). The following are several of the techniques used for purifying algal cultures.

Washing by Centrifugation Technique

Bacteria and algae usually can be separated readily by centrifugation and washing in sterile medium. Fill a sterile, thick-walled, 15-mL centrifuge tube with

algae from a vigorously growing culture. Spin the material at approximately 2000 rpm for 45 to 90 seconds. Decant the supernatant and resuspend the algae in sterile water or medium. Repeat this procedure at least 12 times.

After the last washing, decant the liquid and resuspend the algae in about 1 mL of sterile liquid. With a sterile pipet, place a few drops onto an agar plate (Bristol's agar, proteose agar, soil-extract agar). Streak the material in the form of a pentagon (one side at a time) with a flamed bacteriological transfer loop. At the stopping point of each line, reflame the transfer loop such that only those cells at the end of the previous streak are used for the next streak. After the culture has grown under lights, colonies in the later streaks will be spaced farther apart and will almost always be from single cells. Once colonies are evident, examine the agar plate with a dissecting microscope. With a micropipet or bacteriological transfer loop, move any colonies that show no sign of contamination to fresh agar slants.

Brown and Bischoff (1962) further modified the above techniques to include several treatments of the algal cells with detergent solutions and with short pulses in an ultrasonic water bath (low intensity with a frequency in the range of 90 kc per second) to facilitate algal and bacterial separation. Prior to the centrifugation and washings, treat the algal cells with a synthetic detergent solution or a 5% solution of the nonionic surfactant "Tween 80." Give the cells in detergent solution several ultrasonic vibration treatments, each from 60 seconds to several minutes duration (depending on the organism). Then wash the cells by centrifugation as described above, and resuspend after each washing with 10-second bursts of ultrasonic vibration.

Antibiotics Technique

Antibiotic mixtures can be used successfully to purify cultures of algae that are too large for the centrifuge technique. The following two formulas allow for a variation in approach.

Formula 1

Make a stock solution containing the following:

0.6 g Penicillin "G" (1625 units per mg)
1.0 g Streptomycin Sulfate
200 mL Distilled Water

Filter sterilize the stock. Store the stock in a freezer. To use, add 1 mL of stock to 100 mL of medium. Transfer algae to the medium, leave for 48 hours, and retransfer to fresh, sterile medium without antibiotics.

Formula 2 (Provasoli, 1958)

Make a stock solution such that 1 mL contains the following:

12,000 units K Penicillin
50 µg Chloramphenicol
50 µg Polymyxin B
60 µg Neomycin

Filter sterilize and use 1.5 mL stock per 100 mL medium. Leave the organism in the antibiotic medium for up to 7 days.

Either Formula 1 or Formula 2 may be added to agar media rather than to liquid media.

UV Light Technique

UV light can be used as a selective agent because most algae are more resistant than bacteria to the lethal effects of UV treatment. This technique may be especially effective in purifying algae with a gelatinous matrix that interferes with other purifying techniques. Place a 2750-angstrom UV light about 25 cm above cultures grown on agar or in shallow liquid. Irradiate the cultures for 8 to 16 minutes. Then, transfer individual cells to fresh medium.

Filter Technique

Millipore® or membrane filters can be used to separate filamentous algae and bacteria. Cut filaments into small pieces (one to five cells long) and suspend them in sterile medium in the bowl of a sterilizing filter outfitted with an 8-µm filter disk. Connect the filter to a vacuum source. Continually swirl the filaments to keep them off the filter disk by the addition of 2 L sterile medium in a continual flow. The filter disk must be changed several times during this operation.

Pour the washed pieces of filament directly into a flask of cool but unsolidified Bacto™ nutrient agar. Mix the agar and alga well and pour into sterile petri dishes. Leave the alga suspended in agar for 2 to 3 days. Then, identify pieces of filament that are not surrounded by bacteria, carefully cut them away from the agar, and inoculate them into sterile medium.

Testing for Purity

After a culture has been carried through a purification technique, it should be tested to ensure that it is bacteria-free. The testing is normally done by inoculating at least six standard bacteriological media. Some of the media used for this test are nutrient broth and agar, yeast–dextrose agar, proteose peptone agar, malt agar, sodium caseinate agar, and thioglycollate agar.

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